Evaluation of New Fungicides to Control Citrus Brown Wood Rot – 2019 [1]

Jiahuai "Alex" Hu
School of Plant Sciences, University of Arizona, Tucson, AZ

Introduction

Arizona is one of the largest producers of premium lemons for fresh market in the world. In 2018, a total of 54,000 metric tons of lemons were produced on more than 7,300 acres. In recent years, wood rot diseases have become increasingly important in desert citrus production, with an incidence rate of 70 to 100% in some lemon orchards in Yuma. Typical symptoms of wood rots include bark cracking and splitting, gumming, branch wilt and dieback, wood rot, breakage of scaffold branches, and leaf yellowing and wilting. Although several fungal pathogens such as *Coniophora eremophila*, *Antrodia sinuosa*, *Nodulisporium* sp., and *Fomitopsis meliae* were associated with wood rot (Matheron et al., 2006, Adaskaveg et al., 2014), *F. meliae* is the primary fungal pathogen responsible for brown wood rot. The incidence of BWR caused by *F. meliae* is at all-time high in many lemon orchards in Yuma.

Disease management has been costly and extremely difficult due to limited choices of effective compounds and inability to deliver sufficient dose of fungicides to the infection site, where the fungus resides. Glenn's research (2012) demonstrated that QoI fungicide azoxystrobin and DMI fungicide propiconazole had activities against BWR, but a better application method is needed for adequate control of BWR. Consequently, fungicide evaluations and application method optimization are essential to effective BWR management in view of no current materials labeled for BWR management. The primary objective of this study is to screen the candidate systemic compounds for excellent activities against BWR fungal pathogens and compare the control efficacy of the top 3 performing fungicides by soil drench in greenhouse experiments.

Materials and Methods

Isolation of *F. meliae*. Specimens of plant or fungal fruiting bodies were sprayed with 75% ethanol and washed in running tap water. Bark was first removed to reveal necrotic areas and then wood fragments (7 × 7 mm) were cut from the margin between affected and healthy tissues. These small fragments or fruiting bodies pieces (3 × 3 mm) were surface sterilized by soaking in 75% ethanol for 5 s, 1% sodium hypochlorite for 60 s, copiously rinsed with sterile distilled water, and dried on sterile filter paper in a laminar hood. Each fragment or piece was plated onto malt extract agar (MEA) and water agar (WA) amended with 100 µg/mL streptomycin. Plates were incubated at 25oC in the dark until typical fungal colonies were observed. Fungal colonies were subcultured onto PDA and incubated at 25° for 4 d. Hyphal tip subculture were obtained for each isolate by removing tips of hyphae (3-to-4 cells) from the colony margin and subculturing them onto fresh PDA (McCabe et al., 1999).

***In vitro* fungicide sensitivity testing.** *In vitro* studies were conducted to evaluate efficacy of various fungicides against these fungal isolates and develop baseline sensitivities to these fungicides. A total of twenty-five DMI, SDHI, and QoI fungicides with various modes of actions were evaluated with 12 isolates of *F. meliae*. Tests were conducted on PDA agar amended with fungicide in a 10-fold dilution series ranging from 0.001 to 10 µg/ml and control plates not amended with fungicides. A 5-mm-diameter disk containing mycelium and agar from the margin of actively growing colonies of 4- to 6-day-old cultures were positioned in the center of a culture dish. Isolate growth will be determined by measuring colony diameters in two perpendicular directions after 6 days of incubation in the dark at 30 ± 1ºC. Measurements were averaged, the diameter of the mycelial plug were subtracted, and relative growth reduction for each rate of fungicide was calculated as follows: (100 – [growth with fungicide/growth in control plate] × 100). The EC_{50} relative to the control was estimated by plotting the percentage inhibition against the log-scale of fungicide concentration using drc package, R Project.

[1] The authors wish to thank Ms. Jin Huang for her assistance in completing this project. The authors would also like to thank the Arizona Citrus Research Council for supporting this research. This is a final report for project 2019-04.

***In vivo* evaluation of fungicides.** Top three best-performing compounds were evaluated on three years old Lisbon lemon trees in a greenhouse. Inoculum consisting of autoclaved pieces of wheat grains colonized by BWR pathogens were prepared and placed into wounds in living branches as follows. A vertical hole (6 mm in diameter by 20 mm long) was drilled into each branch to be inoculated. Prior to inoculating branches, four trees will be treated with each fungicide at appropriate dose. The inoculum consisted of sterilized wheat grains on which *F. meliae* will be grown for 20 days at 24°C, Isolates were inoculated on trees by inserting five wheat grains in 1.5 cm deep holes which were covered with wet gauze and mastic. Three branches were inoculated for each tree and each fungicide treatment was replicated with 3 lemon trees. In addition, three trees receiving no fungicide treatment served as a control. Fungicides were applied to pot as soil drench at 10 ppm and trees were inoculated immediately after fungicide treatment. Disease development were assessed approximately four months later, by removing inoculated branches, splitting them in half, and measuring the resultant decay columns.

Results and Discussion

***In vitro* fungicide sensitivity testing.** All fungicides tested, except for Pydiflumetofen, Fluopyram, Thiabendazole, and Pyrimethanil, could reduce fungal growth of *F. meliae* at the concentration of 1 µg/ml. Demethylation inhibitors, except for flutriafol, had lower EC_{50} values than Strobilurins. There were little variations in responses among 12 isolates to various concentrations of a fungicide. Succinate Dehydrogenase Inhibitor fungicides had little inhibitory effect on the mycelial growth of *F. meliae*. DMI and Strobilurins fungicides had lower EC_{50} values compared to other fungicides tested. No inhibition was observed for SDHI fungicides.

Table 1. EC_{50} values of fungicides tested *in vitro* for *Fomitopsis meliae* associated with citrus brown wood rot

Active Ingredient	Trade Name	Manufacturer	EC_{50} Values (µg/ml)*
Tebuconazole	Folicur 3.6 F	Bayer	0.1273 ± 0.0476 a
Difenoconazole+Propiconazole	CGA64250/CGA169374 EC	Syngenta	0.1274 ± 0.0539 a
Azoxystrobin+Benzovindiflupyr	Elatus WG	Syngenta	0.1482 ± 0.0380 a
Difenoconazole+Benzovindiflupyr	Aprovia Top	Syngenta	0.1683 ± 0.1824 a
Difenoconazole+Azoxystrobin	Quadris Top	Syngenta	0.2694 ± 0.6238 b
Fluopyram+Tebuconazole	Luna Experience	Bayer	0.3336 ± 0.0900 b
Fludioxonil	Cannonball	Syngenta	0.4189 ± 0.1717 c
Propiconazole	Mentor	Syngenta	0.6285 ± 0.1790 d
Azoxystrobin+Propiconazole	Quilt Xcel	Syngenta	1.4779 ± 3.1322 e
Azoxystrobin	Quadris	Syngenta	6.8278 ± 1.1213 f
Flutriafol	Rhyme	FMC	7.1355 ± 2.3280 f

*Values were mean of 12 isolates ± standard deviation; Means followed by different letters were significantly different using Tukey's honest significant difference (HSD) (α = 0.05)

***In vivo* evaluation of fungicides.** Mean lesion lengths ranged from 3.8 to 8.2 cm, A significant difference in lesion length ($P < 0.05$) was detected among fungicides (Table 2). Mean lesion length for trees receiving Quadris Top and Quadris were 3.8 and 4.5 cm. These numbers corresponded to 53.6% and 45.1% inhibition for lesion development as compared to that of trees without fungicide treatment. Folicur 3.6F did not reduced lesion size significantly as compared to control. It was likely that Folicur was not absorbed by tree roots and translocated in adequate amount to inoculation sites.

Table 2. 2018 and 2019 Lemon tree height, canopy volume and health rating.

Treatment	Length of wood decay column on inoculated branch (cm)
Non-treated control	8.2 a
Folicur 3.6F, 10 ppm	7.4 a
Quadris, 10 ppm	4.5 b
Quadris Top, 10 ppm	3.8 b

One-way ANOVA were performed to determine the treatment effect. Fisher's least square test was performed to compare treatment means among different fungicides. Column with the different letter indicated significant difference among fungicide treatment

Conclusions

1. Based on our *in vitro* fungicide screening results, there were ten fungicides showing excellent activities with EC_{50} values less than 0.5 ppm. Any fungicide containing propiconazole, difenoconazole, benzovindiflupyr, fludioxonil, azoxystrobin, and trifloxystrobin were highly effective and showed complete inhibition of all the isolates evaluated at concentrations as low as 5 ppm. Other fungicides showed variable responses among the isolates and were not as effective as the ones mentioned above.
2. In our greenhouse experiments, azoxystrobin + propiconazole, and azoxystrobin had the greatest inhibition of wood decay by *F. meliae*. Applied alone, azoxystrobin reduced the disease severity by 45.1%. Soil drench might not be most effective way to apply protective, non-systemic fungicides for control of brown wood rot. Additional research is needed to determine the effective way of delivering fungicides to active infection site.
3. These fungicides should be applied for preventive protection. To maximize their control activity these fungicides may be applied at regular intervals as has been reported in the control of other diseases. Fungicides applied only once may lose their control ability mostly due to their dilution in the plant or tree tissues over time and to the deposition of new spores that can reinforce the infection. Regular prophylactic use of fungicides, especially if combined with sanitation pruning, will result in an appreciable decrease in disease severity. Additional field studies need to be carried out to optimize fungicide application. In particular, epidemiological studies which would improve the timing and frequency of fungicide application would be useful. Furthermore, cultural practices such as pruning and fungicide applications should be investigated more fully as an integrated form of disease management.

References

Adaskaveg JE, F Ö Rster H, Mauk PA, 2014. Fungal diseases. In: Ferguson L, E G-CE, eds. *Citrus Production Manual.* Oakland, CA: University of California, Agriculture and Natural Resources, Publication 3539, 307-26.

Matheron ME, Porchas M, Bigelow DM, 2006. Factors Affecting the Development of Wood Rot on Lemon Trees Infected with Antrodia sinuosa, Coniophora eremophila, and a Nodulisporium sp. *Plant Dis* **90**, 554-8.

Mccabe PM, Gallacher MP, Deacon JW, 1999. Evidence for segregation of somatic incompatibility during hyphal tip subculture of Rhizoctonia solani AG 4. *Mycological Research* **103**, 1323-31.

Supplementary data

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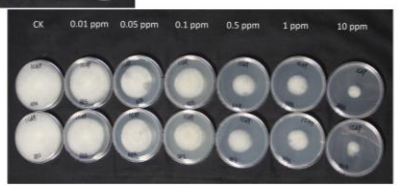
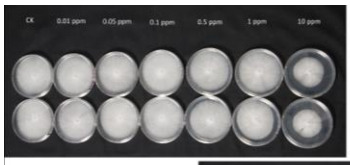


EC_{50} values:

0.53ppm

0.14~0.75ppm



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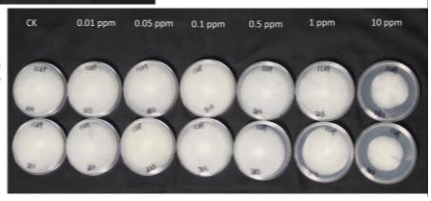

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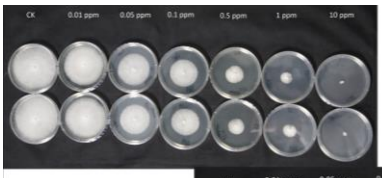

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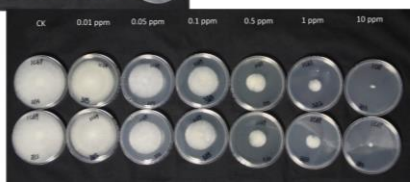
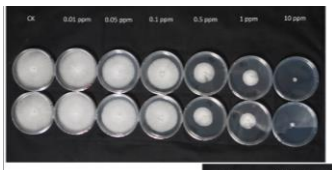


EC_{50} values:

0.45ppm

0.16~0.67ppm



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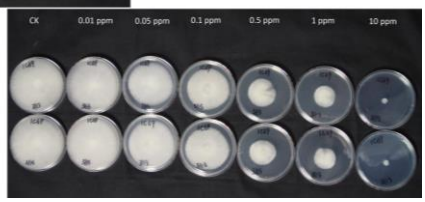
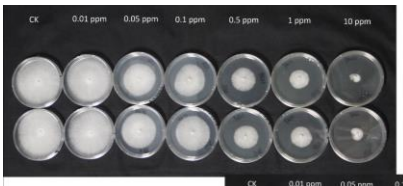


EC_{50} values:

0.82ppm

0.52~1.1ppm



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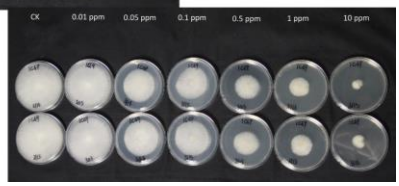
Pristine



EC_{50} values:

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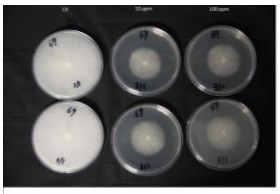
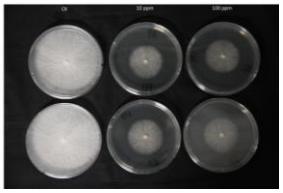
0.19~1.19ppm



Bayer-Flint



EC_{50} values:
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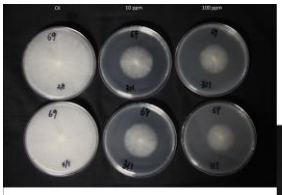
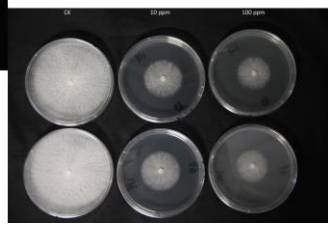
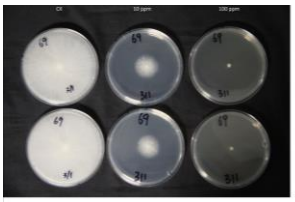


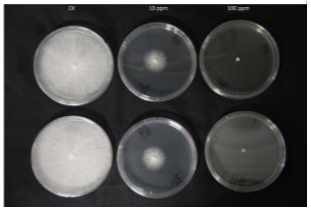
Bayer-Luna sensation



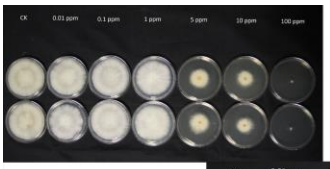
EC_{50} values:
10ppm



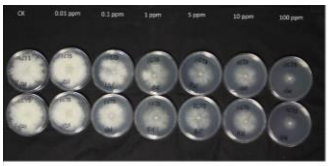
FMC-preemptor



EC_{50} values:
4.82ppm

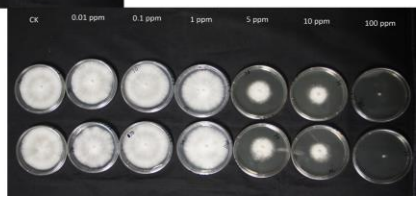


FMC-Rhyme



FMC-TopGuard EQ

EC_{50} values:
15.90ppm



EC_{50} values:
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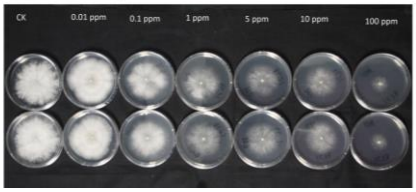
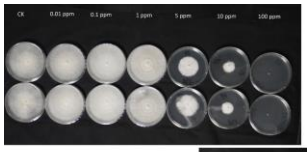
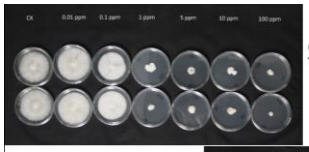


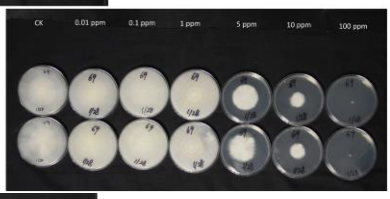


FMC-TopGuard Terra



Syngenta-Cannonball WP

EC_{50} values:
16.34ppm



EC_{50} values:
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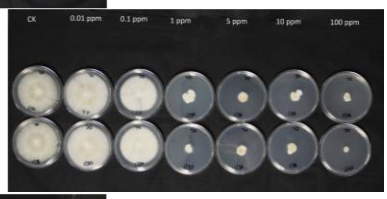
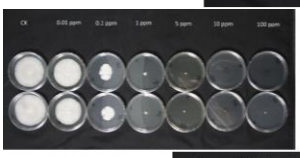
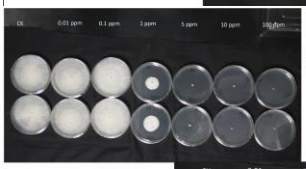




Syngenta-
CGA642501CGA 69374FC



Syngenta-Mentor

EC_{50} values:
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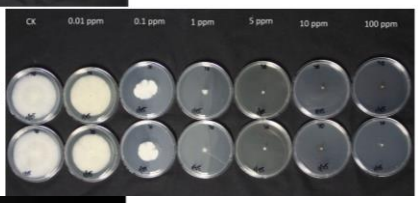

EC_{50} values:
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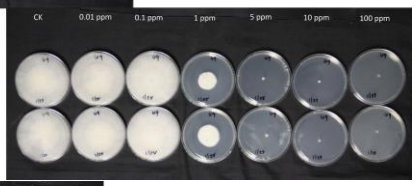
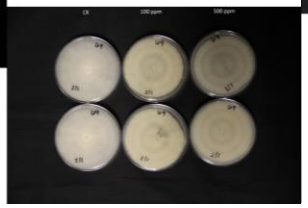


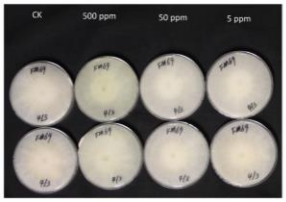
Syngenta-Miravis

Syngenta-Quadris

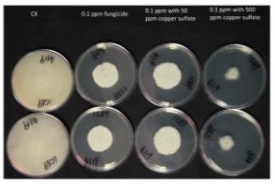


EC_{50} values:
>500ppm

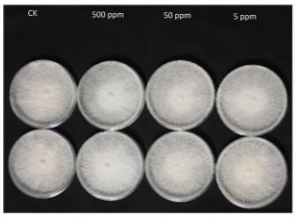
EC_{50} values:
3.82ppm



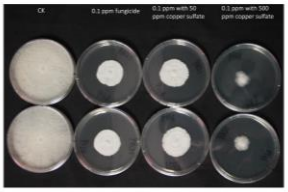
Copper sulfate



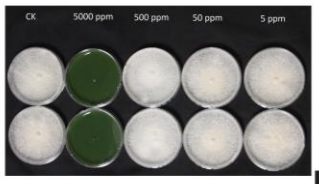
CGA64250-CGA16374EC
+ Copper sulfate



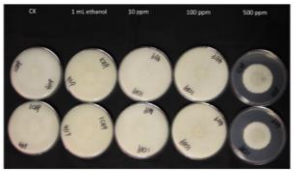
<u>Copper sulfate</u>
No mycelial inhibitory effect at 500 ppm



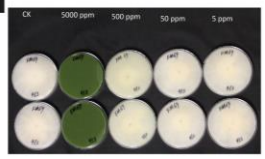
<u>Copper sulfate</u>
If incorporated at 500 ppm, works synergistically with CGA64250



Kocide 3000

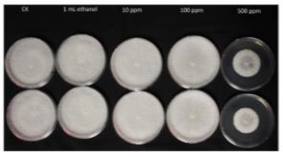


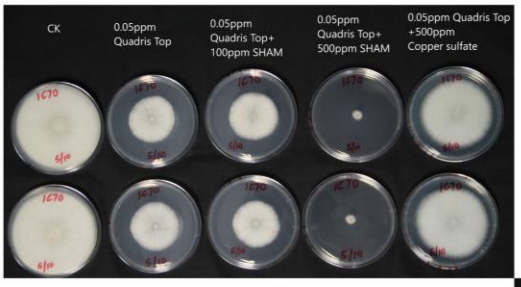
Sham



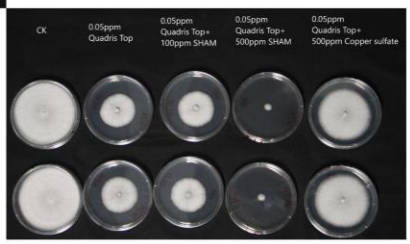
<u>Kocide 3000</u>
No mycelial inhibitory effect at 500 ppm

<u>Sham</u>
No mycelial inhibitory effect when less than 500 ppm
EC_{50} values: 500 pm





Sham+
Quadris Top



<u>Sham @500 pm</u>
Worked synergistically with Quadris Top@0.05ppm